Human exposure to pesticides from food

A pilot study

For Coop Sverige AB

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This report has been reviewed and approved in accordance with IVL's management system

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Summary

The IVL Swedish Environmental Research Institute was commissioned by Coop Sverige AB to conduct a small survey on whether a switch from conventional to organic foods can provide a measurable effect on the level of plant protection products in the body. The study has been conducted using a family with three children who usually do not eat organic food. The family first had to eat conventional, non-organically grown food, followed by only organic food. Urine samples were taken from all family members throughout the period and their pesticide residue content was analysed.

The results of the survey clearly show that some pesticides are absorbed into the body through diet. By choosing organic products, it is possible by and large to avoid the consumption of these chemicals through food. Compared with the period when the family consumed conventionally grown food, the concentrations of pesticide residues decreased on average by a factor of 6.7 when the family ate organic food. The children in particular had lower concentrations during the period of organic food consumption. Levels of most, but not all tested pesticides fell in the adults.

Since 1 September 2008, the same pesticide residue thresholds in or on food apply in the EU. The same thresholds apply to imported food. The Swedish National Food Agency (SNFA) is responsible for conducting annual inspections of pesticide residues in vegetable-based and animal-based foods. The SNFA believes that occasionally eating food containing a substance in excess of the threshold does not normally pose any health risk.

The substance that was found in the highest concentration was chlormequat chloride (CCC) in a sample from the youngest child. Based on the information from the food diary, this could be explained through the child having eaten more grain products – such as porridge, bread, and pasta – compared with the adult family members. Chlormequat chloride is used as a straw-shortening agent in the cultivation of grain.

The concentrations measured in the urine show that although pesticides are present in the body, the levels are low and, when converted, are estimated to be below the ADI value (acceptable daily intake) by a good margin. The ADI value is the maximum quantity of a substance that a person can consume daily throughout his or her lifetime without this posing any risk to their health. It is therefore unlikely that a single substance would pose any risk to humans. That said, the system currently used for risk-assessing chemicals is suitable only for one substance at a time. There is, therefore, no approved method for making an overall assessment of the effect of multiple chemicals simultaneously (i.e. combination effects, popularly known as the "cocktail effect"). There is an awareness that this is a major shortcoming. The environmental quality objectives set by the Swedish Parliament with regard to a "Non-toxic Environment" mean that the amount of such substances in the environment must not threaten it or human health. There is still huge potential for reaching non-toxic environment objectives at the national level.

This study clearly illustrates that the food we eat is one route to exposure to chemicals. This is particularly true for children, who eat and breathe more in relation to their body weight. Consequently, the same exposure results in higher concentrations of chemicals in their bodies than in adults (Swedish Chemicals Agency

2014). Eating organic foods reduces the levels of a number of chemicals and substances that we are exposed to through what we eat. This in turn reduces the risk of a long-term impact and combination effects. Choosing organic products also helps to reduce the spread of chemicals in the environment and protects those who work in the cultivation of fruit and vegetables. That said, man's total chemical burden depends on a number of other lifestyle factors and product choices. In order to conduct a full assessment of how the total chemical burden is affected by food choices, a more comprehensive study is required in which exposure to a greater number of chemical substances is examined in a greater number of individuals.

Glossary

Pesticide	A chemical intended to kill, prevent the growth of, or otherwise control the growth of harmful organisms.
Plant protection product	A pesticide used primarily within agriculture.
ADI	Acceptable daily intake – the amount of a substance that is considered safe to ingest every day with no risk of adverse health effects.
Combination effect	Effect that occurs due to the interaction of chemicals that together give rise to a stronger or weaker effect than they would have individually.
Cocktail effect	Another name for combination effect.
Human exposure	When a person is exposed to a chemical substance, for example through food or the use of products containing chemicals.
Consumption	Intake of food or other foodstuffs (in this context).
Endocrine disruptor	A substance that can affect the body's hormonal balance, e.g. affecting the body's ability to reproduce.
Conventionally grown food	Food not grown organically, often with the use of artificial fertilisers and chemical pesticides.
Organic food	Food grown organically, free from artificial fertilisers and chemical pesticides.
Metabolite	A chemical substance formed when another chemical substance (parent substance) is broken down.

1 Introduction

The IVL Swedish Environmental Research Institute was commissioned by Coop Sverige AB to conduct a survey on how a change in diet to more organic foods has an impact on exposure to plant protection products. The study has been conducted on a family which usually does not eat organic food.

2 Background

Plant protection products are used in agriculture primarily to protect crops from fungal attacks, insects, and competing plants, as well as to affect the plant's appearance.

The active substances that may be present in a pesticide are approved at EU level, but the products themselves must be approved in each member state. In order for the use of a plant protection product to be permitted in Sweden, the substance must undergo licence testing at the Swedish Chemicals Agency. Approval is made after consultation with SNFA, among others. This procedure means that there can be considerable differences between EU member states in terms of which products are approved for use on the same fruit or crop. A substance that is not approved in Sweden does not necessarily mean that it is dangerous. It may simply mean that no company has applied for a licence to use the substance in Sweden. Since 1 September 2008, the same pesticide residue thresholds apply in the EU, regardless of whether the pesticide penetrates the food or is superficial. The same thresholds apply to imported food. The threshold is the maximum permissible amount of a substance (mg/kg) in a given food and must take the safety of all consumer groups into consideration. This includes infants, children, and those who consumer a high quantity of fruit and vegetables, such as vegetarians. The thresholds are determined for different active substances and for different products, which means that a threshold for a given substance may differ between an apple and an orange, for instance.

The Swedish National Food Agency (SNFA) is responsible for conducting annual inspections of pesticide residues in vegetable-based and animal-based foods. The results of the 2011-2012 survey show that pesticide residues were found in 86% of fruit samples and in 46% of vegetable samples (Fohgelberg et al. 2014).
 However, there were few samples that exceeded the thresholds. Of 3,313 spot checks of fresh, frozen, or processed vegetables, fruit, grain products, and animal products, 106 samples (3%) exceeded the EU's thresholds. Most threshold exceedances were reported in vegetables – 54 out of 954 samples (6%). A report by SNFA shows that Swedish foodstuffs contain fewer pesticide residues than imported foodstuffs, and that foodstuffs from countries outside the EU are more likely to contain pesticide residues than foodstuffs from within the EU (Wannberg et al. 2013). It is also more common for foodstuffs from outside the EU to exceed the established thresholds.

The primary exposure to plant protection products for people who do not handle them in their profession is through food (Lu et al. 2001, 2006). How much plant protection product a person is exposed to through food depends on their choice of food and how much of that food the person eats.

The SNFA believes that occasionally eating food containing a substance in excess of the threshold does not normally pose any health risk since the threshold includes a safety margin. For acutely toxic substances, the safety margin may be less, especially for children. In a summary of the results of surveys carried out in various European countries in 2010, 79 of a total of 18,243 samples (0.4%) indicated a potentially acute risk of pesticide residues (EFSA 2013).

A compilation of information from several studies made by Lund University shows that plant protection product residues are more prevalent in conventionally grown food than in organically grown food (Buchholt and Persson 2006). Unlike conventional crops, chemical pesticides are not allowed to be used on organic crops. A study of urine samples from 100 people in Skåne, Sweden showed higher levels of pesticides in people who usually do not eat organic products compared with those who preferred organic produce (Littorin et al. 2005). Another study has shown that children who eat organically grown food have less exposure to pesticides (Lu et al. 2006). Oates et al. (2014) showed that the levels of organophosphate-based plant protection products decreased by 89% in 13 adults after eating organic food for one week.

The Department of Occupational and Environmental Medicine at Lund University was commissioned by the Swedish Environmental Protection Agency (SEPA) to conduct studies of human exposure to pesticides by measuring the levels of pesticide residues in the urine of various groups of the population. These studies concluded that certain plant protection product residues were present in 90%-100% of participants (Littorin et al. 2009; Littorin et al. 2013).

3 Methodology

The present study involved a family comprising two adults (aged 40 and 39) and three children (aged 12, 10, and 3), all of whom usually eat conventionally grown food. The experiment began with the family eating conventionally grown food for one week. In the following two weeks the family ate organic food, during which time all food was organic – fruit, vegetables, meat, fish, etc.

Other household products such as personal hygiene products, detergents, and new textiles may have contained the antibacterial agent triclocarban. When triclocarban degrades in the body, the metabolite 3,5-dichloroaniline (3,5-DCA) is formed, which is also a degradation product of pesticides (see Table 1). Consequently, it was important to take this form of exposure into account during the weeks when the family was consuming organic food so as to facilitate the evaluation of this study. Consequently, detergents were changed during the organic weeks. The family was also asked not to wear newly purchased clothing, bedding, or towels during this period. The father of the family uses snus, which was replaced with organic snus during the organic weeks. The family was already using environmentally friendly personal hygiene products such as shampoo, conditioner, and skin care products prior to the study.

3.1 Sampling

Urine was collected every morning throughout the experiment, both in order to establish a routine for the convenience of the family and to have a larger range of samples to analyse. One of the children was in nappies at the start of the experiment, which meant that the time of the sampling varied from day to day. The family wrote a diary of what they ate each day. Urine samples from the first and last week were analysed (Figure 1). Based on the information from the food diaries, four urine samples per person, per week were analysed (i.e. eight samples per person, or a total of 20 urine samples from when the family ate conventional food, and eight samples per person, or a total of 20 urine samples from when the family ate organically grown food).

	Family eats as normal	Family eats organically grown food	
Period:	Period 1	Period 2	
Week:	Week 1	Week 2	Week 3
Day:	1 2 3 4 5 6 7	8 9 10 11 12 13 14	15 16 17 18 19 20 21
	Sampling		Sampling

Figure 1. Implementation of sampling.

3.2 Analysis

All urine samples were analysed for the presence of 12 different pesticide residues (Table 1) at IVL's laboratory in Stockholm. Substances were selected based on previously reported experiences of which substances had been found in various foods and in urine samples from humans (Littorin et al. 2009; Littorin 2011). The methods of analysis are reported in Appendix A. Detailed information about the analysed substances can be found in Appendix B, Table B1.

Table 1. Pesticide residues analysed in morning urine. Where a metabolite (degradation product) is indicated, this has been analysed instead of the original substance.

Pesticide	Metabolite	Function	Found in e.g.:
MCPA		Herbicide	citrus fruits
Ethylenebisdithiocarbamates	ETU	Fungicide	wine, grapes, and raisins
Atrazine		Herbicide	herbicide against weeds
Chlorpyrifos	3,5,6-Trichloro-2-pyridinol	Insecticide	wine, grapes, raisins, oranges
Thiabendazole		Fungicide	apples, pears, oranges
For example, iprodione, diuron, vinclozolin	3,5-DCA	Fungicide	lettuce, wine, grapes, tomatoes
Boscalid		Fungicide	tomatoes, strawberries
2,4-dichlorophenoxyacetic acid (2,4-D)		Herbicide	herbicide against weeds
Pyrethroids, such as cypermethrin and esfenvalerate	3-PBA	Insecticide	grain, fruit, and vegetables
Propamocarb		Fungicide	cucumber, lettuce
Chlormequat chloride (CCC)		Straw-shortening agent (growth	grapes, grain products
Mepiquat		Straw-shortening agent (growth inhibitor)	grain products, coffee

3.3 Dilution effects

The concentration of chemicals and other substances in the urine is, in addition to the intake of the substance in question, largely dependent on how diluted the urine is, which in turn depends on how much the person has eaten and drunk before the sample was taken. To enable comparisons between concentrations at different times of day, on different days, and between individuals, it is necessary to take these dilution effects into account in the presentation of the results. Various methods are used when reporting concentrations in urine, such as density adjustments or adjustment for creatinine content. Studies have compared the different methods and these are generally considered to be equivalent (Haddow et al. 1994). In this study, we have adjusted the concentrations based on the creatinine content by dividing the measured concentration of a substance by the measured concentration of creatinine in the urine, resulting in a concentration expressed in µg/g creatinine (µg/g crt). Carrieri et al. (2000) developed a correlation factor of 1.48 to compare different normalisation methods with each other. Where other studies have used density-adjusted values, we have therefore: converted these as follows: $C_{,density} (µg/L) \times \dfrac{1}{1.48} = C_{creatinine} (µg/g \, crt)$, to compare with levels measured in the present study.

4   Results and discussion

The results from the survey show that exposure to pesticides was reduced when the family switched from conventional to organic food (Figures 1 to 5; note the different scales on the y-axis for the different family members). On average, the concentrations decreased by a factor of 9.5 during the period of organic food. On average, the decrease was slightly higher in the children (factor 12) than in the adults (factor 9), yet the youngest child and the mother have the largest decreases (factors 27 and 25 respectively). The greatest decrease was observed for 3,5-DCA (factor 22), CCC (factor 18), and 3-PBA (factor 15), but only a limited difference (factors 2-3) was indicated for ETU, propamocarb, and 2,4-D. While the children and the mother showed lower concentrations of TCP and mepiquat during the period of organic food, the father showed concentrations of these substances that were just as high or higher than during the period of conventional food. In earlier studies, these pesticide residues have been linked to the consumption of conventionally grown wine (TCP) and coffee (mepiquat).

Below is a summary of the results from the study. All the results are presented in Appendix B, Table B2. All of the concentrations in the report are normalised by creatinine. In calculating the median, values below the detection limit have been regarded as equivalent to half the detection limit.
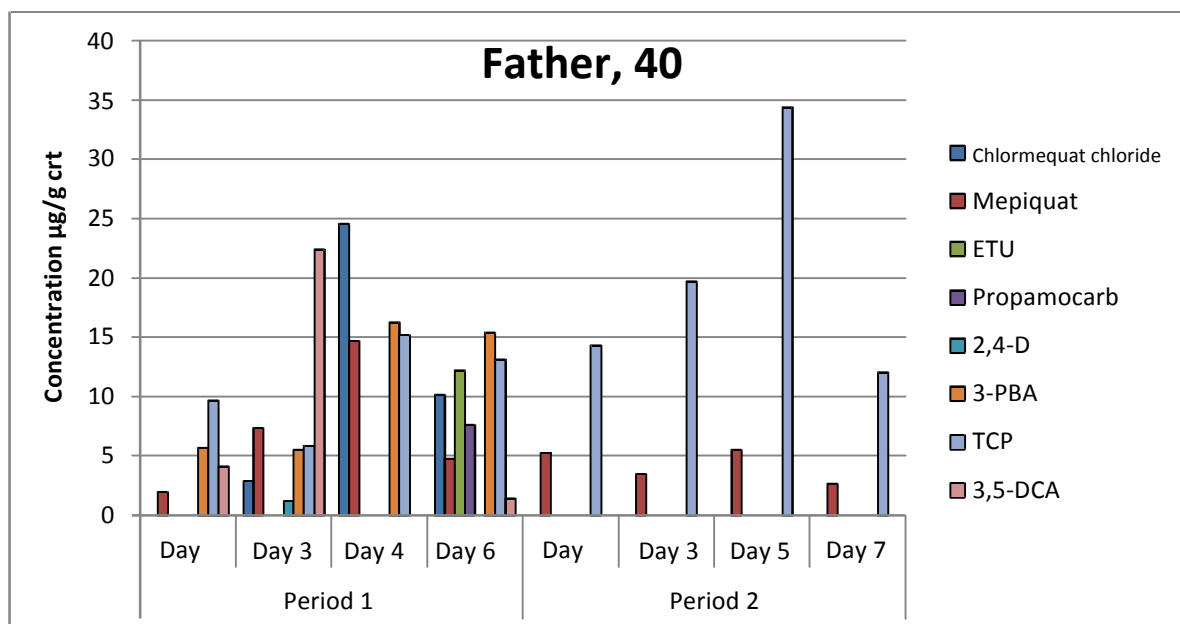

Figure 1. Concentration of pesticide residues in urine samples from the father during the periods of conventional and organic food.
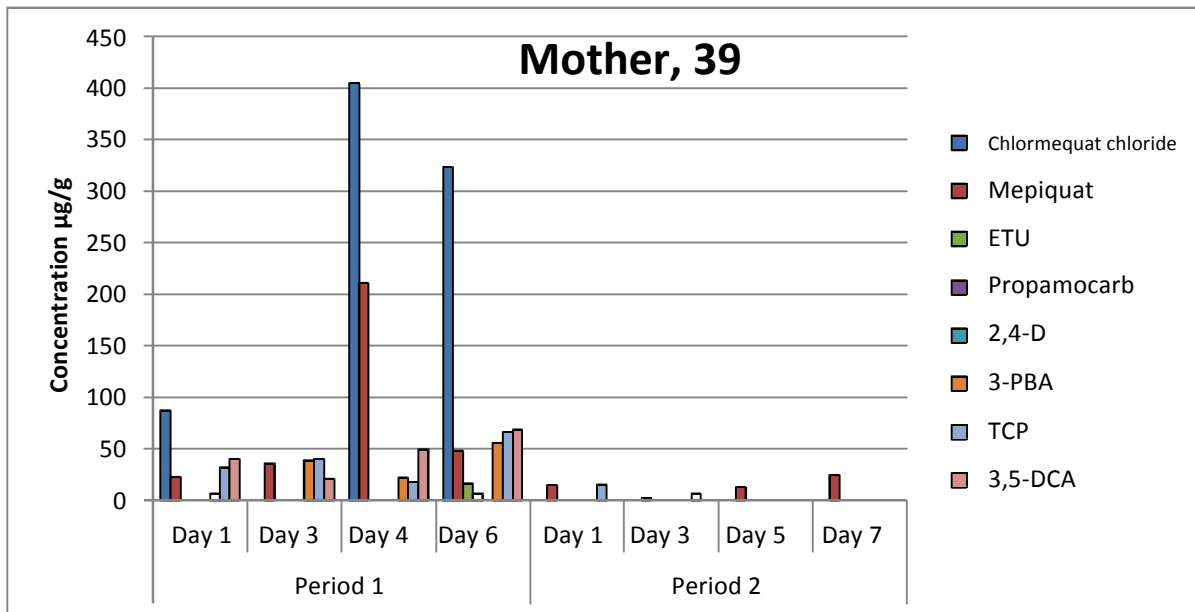
Figure 2. Concentration of pesticide residues in urine samples from the mother during the periods of conventional and organic food.
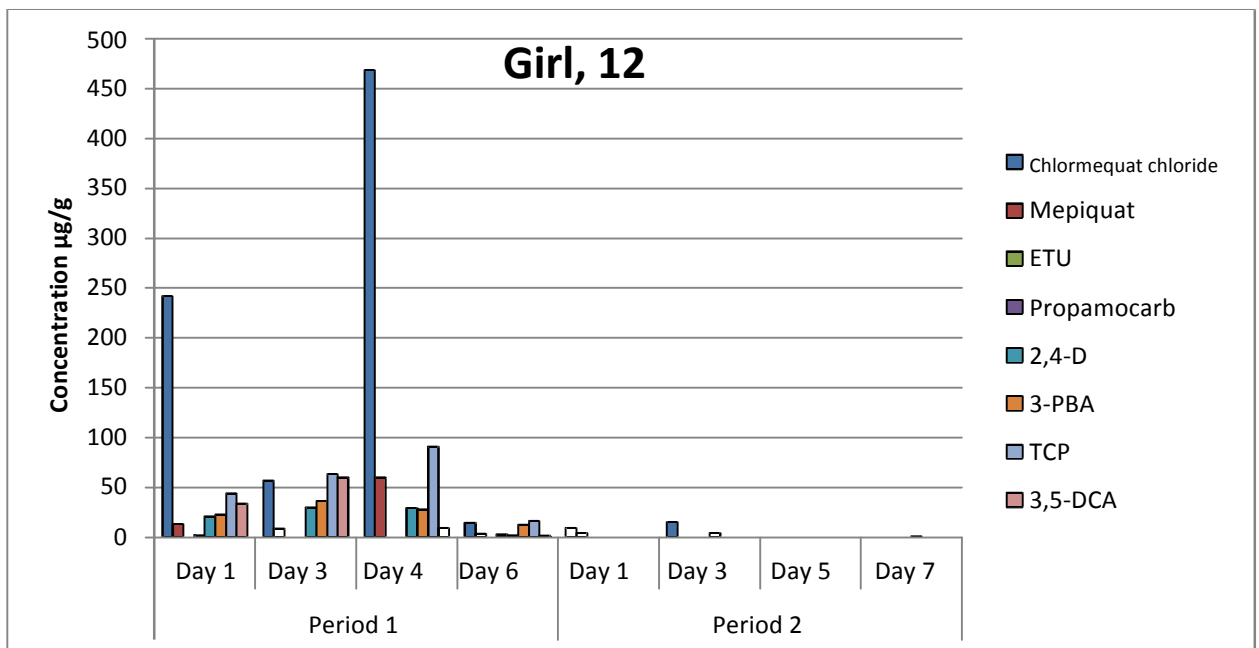

Figure 3. Concentration of pesticide residues in urine samples from the eldest child during the periods of conventional and organic food.
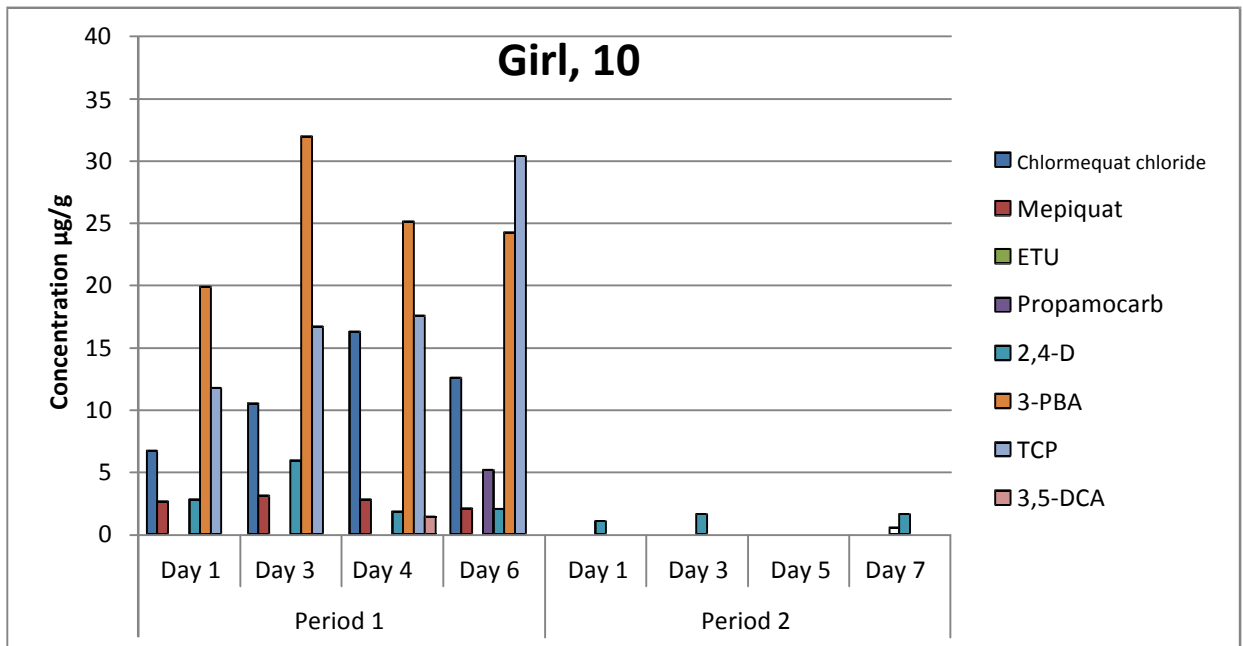
Figure 4. Concentration of pesticide residues in urine samples from the middle child during the periods of conventional and organic food.
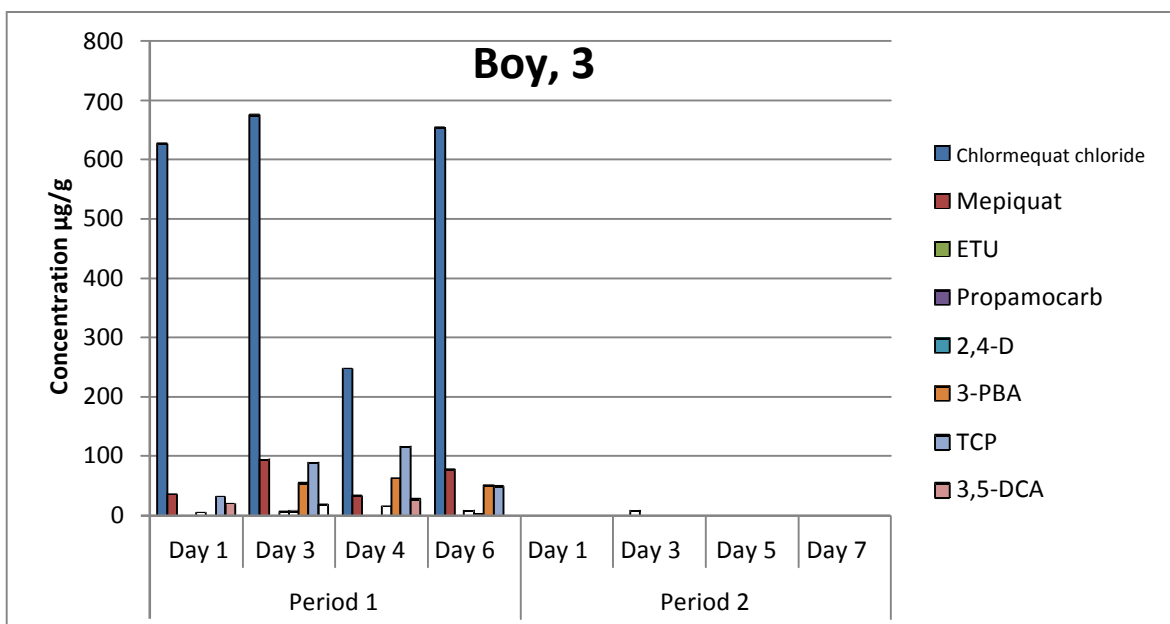

Figure 5. Concentration of pesticide residues in urine samples from the youngest child during the periods of conventional and organic food.

4.1 Experiment period with conventional food

During the period when the family ate conventionally grown food, 8 out of the 12 analysed pesticide residues were found in at least one of the urine samples (Table 2). In two of the children the median level was over the detection limit for 7 pesticide residues, and for one of the children and both adults the median level was over the detection limit for 5 different pesticide residues.

Table 2. Pesticide residues found in urine samples after consumption of conventionally grown food. Values are adjusted for creatinine content and are stated in μg/g crt. The comments describe in which family members the analysed substances were found.

Pesticide	Metabolite	Median (μg/g crt)	Interval (μg/g crt)	Detection frequency	Detection limit (μg/g crt)	Comments
Ethylenebisdi-thiocarbamates	ETU	<LOD	<LOD – 17	2/20	0.55-6.7	Only in adults
Chlorpyrifos	TCP	31	6-116	20/20	1.3-17	In all family members
For example, iprodione, diuron, vinclozolin	3,5-DCA	13	<LOD-69	12/20	0.5-5.6	Generally occurring in all people except one child
2,4-D		2.0	<LOD-29	13/20	0.25-3.0	All samples from the children and a sample from an adult
Pyrethroids such as cypermethrin and esfenvalerate	3-PBA	24	<LOD-63	19/20	1.2-15	In all family members
Propamocarb		<LOD	<LOD-8	7/20	0.11-1.4	In individual samples all family members
Chlormequat chloride (CCC)		41	<LOD-675	18/20	1.2-15	In all family members
Mepiquat		14	2-211	20/20	0.95-12	In all family members

4.1.1 The presence of pesticide residues in urine samples

TCP was found in all 20 samples at concentrations of between 6 and 116 μg/g crt. TCP is a degradation product (metabolite) of chlorpyrifos, an insecticide used in the cultivation of fruit and vegetables. Since 2008, there have been no approved herbicides on the Swedish market that contain chlorpyrifos, although there are within the EU (EU pesticide database). Data does indicate its widespread use, however. In a study of pesticide residues in urine samples from 128 women conducted in Skåne, Sweden in 2010, TCP was found in all samples and in higher concentrations in vegetarians than non-vegetarians (Littorin et al. 2013). According to studies by SNFA, chlorpyrifos is one of the most prevalent of the pesticide residues in citrus fruits (Fohgelberg et al. 2014). TCP has also been measured in urine in an earlier study in concentrations of up to 4.3 ng/ml (Littorin et al. 2011), which with a correlation factor of 1.48 corresponds to 2.9 μg/g crt, i.e. slightly lower than in this study.

Mepiquat was found in all 20 samples. The highest concentrations of mepiquat were found in the youngest child (median: 57 µg/g crt) which, based on information in the food diary, could be explained by a higher intake of several different grain products. The substance also occurred in the other two children, albeit at lower concentrations. The occurrence of mepiquat in the parents' urine could be explained by the fact that both of them drank coffee during the period, and the father also used snus. Earlier studies have linked mepiquat to the consumption of coffee and smoking of tobacco (Littorin et al. 2013). According to the food diary, the mother also consumed significantly more coffee, which is also reflected in her higher concentration of mepiquat compared with the father. On one day, it is noted that the mother drank 10 cups of coffee. Concentrations of mepiquat in samples from that morning and the following morning (i.e. before and after consumption) increased from 36 to 211 µg/g crt, which was the single highest value measured among all samples. Mepiquat is a growth inhibitor used on grain and in coffee plantations. The substance is approved for use in Sweden.

Chlormequat chloride (CCC) (which is mainly used in the cultivation of grain) was found in 18 of the 20 samples analysed. Concentrations ranged between 2.9 and 675 µg/g crt (median: 41 µg/g crt). The highest measured concentrations of CCC were found in the youngest child (median: 640 µg/g crt), which could be explained by the fact that this child consumes more grain products in relation to his body weight than the other family members. The food diary supports this to some extent. Like mepiquat, CCC is a growth inhibitor used in, e.g., the cultivation of grain as a straw-shortening agent so that the grain remains upright. There is only one approved preparation containing CCC in Sweden. According to the European Food Safety Authority (EFSA), CCC is the most common pesticide residue found in oats and grapes from India (EFSA 2010). Littorin et al. (2011) have previously demonstrated CCC in urine in density-adjusted concentrations of up to 19 ng/mL, corresponding to approximately 13 µg/g crt, which, although in the same interval, is slightly lower than the median concentration in this study.

3-PBA was found in 19 of the 20 samples analysed at concentrations of between 5.5 and 63 µg/g crt. 3-PBA is a degradation product of several pyrethroids used as insecticides both in agriculture and in the home. In earlier studies, 3-PBA has been linked to a high intake of fruit and berries (Littorin et al. 2013). It is suspected that high exposure to pyrethroids may have endocrine disrupting effects (Forslund 2012). Density-adjusted concentrations of 3-BPA of up to 4 ng/mL (equivalent to approximately 2.7 µg/g crt) were measured in an earlier Swedish study (Littorin et al. 2009), i.e. just below the concentrations determined in this study.

3,5-DCA was found to be above the detection limit in 15 out of the 20 samples in concentrations between 1.4 and 69 µg/g crt in almost all samples from all family members, apart from the 10-year-old child. One possible explanation is that this child did not eat all the vegetables that the rest of the family did. 3,5-DCA is a metabolite from several different types of fungicides, such as iprodione, vinclozolin, and procymidone, used in the cultivation of fruit and vegetables such as lettuce, tomatoes, and grapes. However, none of these plant protection products are approved for use in Sweden by the Swedish Chemicals Agency (pesticides register) and only one (iprodione) is approved in some EU countries (EU pesticides database). Since the origin of the vegetables eaten by the family during the period is unknown, it is not possible to determine the parent substance.

In an earlier study, the consumption of grapes/raisins has been linked to concentrations of 3,5-DCA (Littorin et al. 2009), although the food diary states that everyone in the family ate grapes or drank wine. 3,5-DCA has previously been found in urine in concentrations of up to 30 ng/ml DJ (Littorin et al. 2009), which is equivalent to approximately 20 µg/g crt – roughly the same concentrations as in this study.

2,4-D was found to be above the detection limit in 13 out of the 20 samples in concentrations between 1.2 and 29 µg/g crt and, apart from one sample from an adult, exclusively in the children. The food diary has not helped us to find an explanation for this difference. An earlier study found high concentrations of 2,4-D in leafy vegetables such as lettuce and spinach, fruit, and berries. It is possible that the children have been exposed to 2,4-D via imported fruits and vegetables, as this herbicide is not approved for use in Sweden (pesticides register) but is approved in several EU countries (EU pesticides database). It is used in field crops and in the cultivation of fruit and vegetables. 2,4-D is used directly on the fruit in the cultivation of citrus fruits in some warmer countries to regulate growth and ripeness (Littorin et al. 2009). 2,4-D was also detected in an earlier study in urine in density-adjusted concentrations of up to 11 ng/ml (Littorin et al. 2009), which is comparable with the concentrations we have measured (i.e. corresponds to approximately 7.4 µg/g crt with a correlation factor of 1.48).

Propamocarb was found in seven of the samples analysed in concentrations of between 2.2 and 8.0 µg/g crt. Propamocarb is used in the cultivation of vegetables with two products approved for use in Sweden.

ETU was found in two of the samples analysed, with the highest measured concentration being 17 µg/g crt. ETU is a degradation product of ethylenebisdithiocarbamates (such as mancozeb and maneb), which have been used against blight in potatoes, for instance. Only one plant protection product containing ethylenebisdithiocarbamates is approved for use in Sweden (pesticides register). Earlier studies have linked higher concentrations of ETU to the consumption of wine in the days prior to sampling (Littorin et al. 2009). ETU is a suspected carcinogen (Swedish Chemicals Agency 2008a). Previously, density-adjusted concentrations of up to 9.4 ng/ml (equivalent to 6.4 µg/g crt) have been measured in urine (Littorin et al. 2009).

4.1.2 The significance of food choice in exposure to pesticide residues

The differences between the different family members in terms of the presence of pesticides in their urine as demonstrated may, in some cases, be explained using the food diary. As seen in the examples above, there is some evidence that the high concentrations of CCC in the youngest child may be associated with a high consumption of grain in relation to his body weight. Similarly, the food diary supports, to some extent, the mother's high concentration of mepiquat on one occasion, which may be due to a high level of coffee consumption the day before the sample. The food diary's structure does not allow for far-reaching conclusions with regard to the relationship between food choices and concentrations. More details are required in order to make such comparisons, such as the exact quantity and origin of the ingredients consumed.

4.2 Experiment period with organic food

Compared with the period of consumption of conventionally grown food, both the levels and number of pesticide residues detected fell during the period the family ate organic food (Figures 1 to 5; note the different scales on the y-axis for the different family members). During this period, 5 out of the 12 analysed pesticide residues were found in concentrations above the detection limit in at least one urine sample (Table 3). For the youngest child, the median was below the detection limit for all of the analysed substances. For the parents, the levels of TCP and mepiquat did not change compared with the conventional food period. These pesticide residues can be linked to the consumption of wine and coffee, despite these being organic products.

Table 3. Pesticide residues found in urine samples after consumption of organically grown food (µg/g crt). The extent to which the pesticide residues were present in the children and/or adults is stated in the comments column.

Pesticide	Metabolite	Median (µg/g crt)	Interval (µg/g crt)	Detection frequency	Detection limit (µg/g crt)	Comments
Chlorpyrifos	TCP	<LOD	<LOD -34	6/20	1.3-13	Only in adults
2,4-D		<LOD	<LOD -4.1	5/20	0.2-2.4	In two of the children
Propamocarb		<LOD	<LOD -0.6	1/20	0.1-0.5	One child
CCC (Chlormequat chloride)		<LOD	<LOD -15	2/20	0.8-12	One child
Mepiquat		3.6	<LOD -25	10/20	0.8-9.5	In all samples from the adults and in one sample from both the eldest and youngest child

4.2.1 The presence of pesticide residues in urine samples after organic consumption

The most noticeable change in concentration in urine after organic consumption was observed for **chlormequat chloride, CCC**, whose median value (all samples) decreased from 41 µg/g crt to below the detection limit. After the organic period, the substance could only be detected in two urine samples from one of the children with the highest concentration being 15 µg/g crt, compared with 675 µg/g crt during the period of conventionally grown food.

2,4-D (herbicide) was only found in 5 samples following the organic period, compared with 13 samples during the first period, with detectable levels in only two of the children. The median fell from 2 µg/g crt to below the detection limit. The highest measured concentration was similar during the two periods, 1.7 ng/ml compared with the previous 2.2 ng/ml.

The detection rate of **propamocarb** decreased from 7/20 to 1/20 and was found in only one sample from one of the children after the organic period, and then only at a concentration close to the detection limit.

Mepiquat was found at levels above the detection limit in all samples from the parents, and in one sample from the youngest child. The highest levels were recorded in samples from the mother (median 14 μg/g crt, compared with 4.4 μg/g crt in the father). One difference between the adults and the children in food consumption was that the parents drank coffee, beer, and wine. As noted in the food diary (see section 4.1.2), the higher frequency of samples with mepiquat from the adults could probably be explained by the consumption of coffee. This would then mean that the coffee, despite its organic labelling, contained mepiquat. To investigate whether this is the case, additional analyses of organic coffee would be required.

TCP was found in all samples from the father and two samples from the mother, but in no samples from the children. The concentrations in the father's urine were even slightly higher than during the period of conventionally grown food (17 and 11 μg/g crt respectively). Earlier studies have found a link between the consumption of wine and/or grapes in the days prior to sampling, and the presence of TCP in urine samples (Littorin et al. 2011; Lövendahl and Arvin 2013), which is explained by its half-life of 27 hours (Morgan et al. 2005). It is unclear whether the organic wine the father drank during the period according to the food diary contained TCP. As with mepiquat and coffee, additional analyses of the wine would be required to investigate whether the levels of TCP in the father's urine can be explained by wine consumption.

4.3 Risk assessment

4.3.1 Estimated daily intake

The acceptable daily intake (ADI) value is the maximum quantity of a substance that a person can consume daily throughout his or her lifetime without this posing any risk to their health. ADI is based on animal studies and represents the highest dose that causes no adverse effects in the most sensitive species. To account for differences in sensitivity within and between species, a safety factor (usually 100) is used by which the value produced is divided. Details about current ADI values for plant protection products in the EU can be found in the EU's pesticide database.

The daily intake of pesticides can be estimated based on measured concentrations in urine (Mage et al. 2004 and Remer et al. 2002). Based on these equations, we have estimated the daily intake of pesticides for the period of conventionally grown food – see Appendix C for calculation methods. Concentrations below the detection limit have been regarded as an interval between zero and the detection limit, and we have thus been able to calculate a median value of the intake per person, as well as a measure of variability based on both the different measurements during the period, and on the detection limit value. ADI is usually stated in milligrams per kilo of body weight per day (mg/kg/d). In Table 4, we have converted the values to μg per kg of body weight per day (μg/kg/d) to make it easier to compare the figures. Figure 6 shows the ratio between the estimated intake and the acceptable daily intake.

Estimates show that the intake is below the ADI for all pesticide residues (Figure 6). The highest daily intake (median) was estimated for chlormequat chloride (CCC) and relates to the mother (4.08 μg per kg body weight per day). This value is 10 times below the ADI. The single highest estimated daily intake for CCC relates to the 12-year-old girl (9.7 μg per kg body weight per day), and corresponds to an intake that is five times lower than the ADI.

Table 4. Estimated daily intake (μg per kg body weight per day). The value stated is the median value, with the minimum and maximum estimated daily intake stated in parentheses. The estimates are based on four samples per individual and substance. Levels below the detection limit have been deemed to correspond to half the detection limit. Where all levels were below the detection limit for an individual, this is marked with an asterisk (*). At the very bottom is the ADI – the amount of a substance that is considered safe to ingest every day with no risk of adverse health effects.

Individual, age (years)	Chlorpyrifos (TCP)[1](μg/kg/d)	2,4-D (μg/kg/d)	CCC (μg/kg/d)	Propamocarb (μg/kg/d)	ETU (μg/kg/d)	Mepiquat (μg/kg/d)	3-PBA[2] (μg/kg/d)	3,5-DKA[3] (μg/kg/d)
Father, 40	0.3 (0.15-0.40)	0.01 (0-0.03)*	0.17 (0-0.64)	0.01 (0-0.2)	0.03 (0-0.32)	0.16 (0.05-0.38)	0.28 (0.14-0.42)	0.07 (0-0.58)
Mother, 39	0.72 (0.35-1.31)	0.01 (0-0.04)*	4.08 (0-8.0)	0.01 (0-0.14)	0.04 (0-0.33)	0.83 (0.45-4.18)	0.6 (0.13-1.1)	0.89 (0.41-1.4)
Girl, 12	1.1 (0.33-1.87)	0.52 (0.04-0.61)	3.1 (0.29-9.7)	0.02 (0-0.05)	0.04 (0-0.12)*	0.22 (0.07-1.24)	0.52 (0.25-0.74)	0.44 (0.03-1.24)
Girl, 10	0.35 (0.24-0.63)	0.05 (0.04-0.12)	0.24 (0.14-0.34)	0.01 (0-0.11)	0.04 (0-0.07)*	0.03 (0-0.11)	0.51 (0.41-0.66)	0.02 (0-0.05)
Boy, 3	0.19 (0.08-1.7)	0.02 (0.01-0.04)	1.7 (0.66-1.8)	0.01 (0-0.02)	0.01 (0-0.02)*	0.15 (0.09-0.25)	0.14 (0-0.17)	0.05 (0-0.07)
ADI	10	50	40	290	2	200	60 and 20 respectively	50

[1]The value has not been corrected as 70% is excreted as TCP.

[2]More pesticides are possible. ADI has been stated for cypermethrin and esfenvalerate.

[3]More pesticides are possible. ADI has been stated for iprodione.
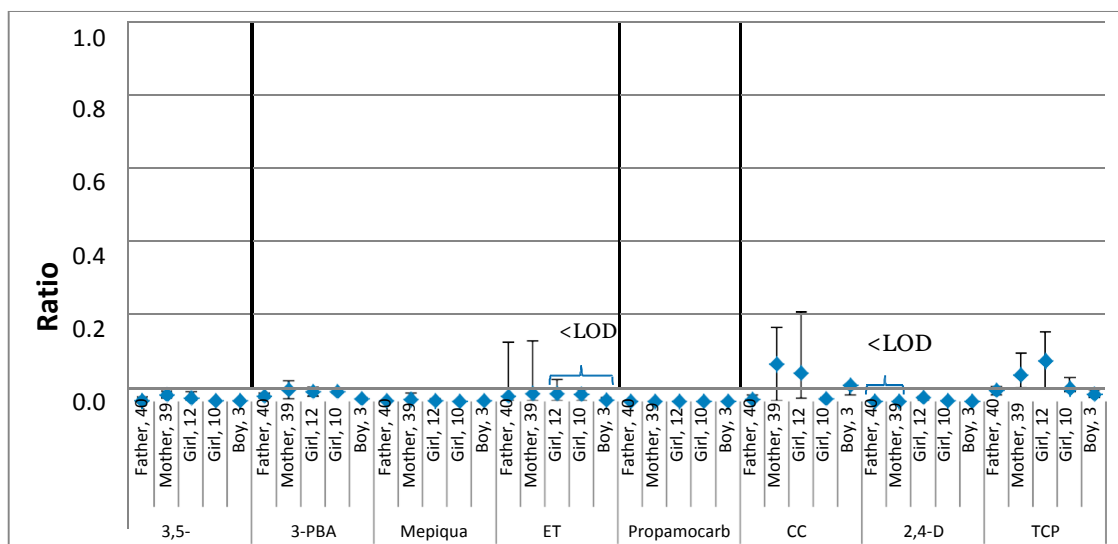
Figure 6. The ratio between the estimate daily intake (EDI) and acceptable daily intake (ADI) of measured pesticides. A ratio of <1 means that the estimated intake is lower than the acceptable intake, i.e. no risk. In calculating the median value, values below the detection limit have been regarded as half the detection limit. Where all values were below the detection limit for an individual, this has been marked in the figure with <LOD.

4.3.2 Risk of combination effects

The levels that we found in urine during the period of conventionally grown food are well within acceptable levels, which means that it is unlikely that a single substance would pose any risk to humans. That said, the system for risk assessing chemicals is suitable only for one substance at a time. There is, therefore, no approved method for making an overall assessment of the effect of multiple chemicals simultaneously, i.e. the possibility that chemicals interact with one another to give a stronger or weaker effect than they would have individually. This is commonly called the "combination effect" or popularly known as the "cocktail effect". This shortcoming has been recognised within the EU and efforts are underway to develop models that better assess the risk of such combination effects (for pesticides see e.g. EFSA 2013).

There are currently limited studies that have looking at combination effects, in part because it is difficult to design such studies with a high degree of reliability. A Danish study (Wohlfahrt-Veje et al. 2011) carried out on children whose mothers worked in greenhouses during the first two months of pregnancy showed that these children had a lower birth weight and higher proportion of body fat between the ages of 6 to 11 than the corresponding control group. In addition, girls reached puberty earlier than in the control group, and between the ages of 6 to 11 had statistically significantly poorer language development, poorer long-term memory, and slower motor skills. These effects were observed despite the fact that only approved pesticides were used, that all work

safety measures were followed, and that the mothers – in accordance with applicable legislation – had to stop working with pesticides when they found out they were pregnant. Despite very detailed information about exposure, the authors could not identify which groups of pesticides were the primary cause, and the underlying mechanisms for the observed effects are unknown. The authors also point out that the products also include other chemicals, such as surfactants and spreading agents which could also result in or contribute to effects.

It should be stressed that there is a big difference between being exposed during early gestation through direct chemical exposure, and indirect exposure through maternal food intake, or in infancy through their own food intake. The levels the greenhouse workers were exposed to are significantly higher than the levels of pesticides that we consume through our food, but the Danish study still clearly demonstrates shortcomings in our current system for the risk assessment of chemical substances.

Even in the external environment, there are indications of the presence of combination effects. Rundlöf et al. (2012) demonstrated, for example, that fungicides can increase the toxicity of insecticides on honeybees.

Given how little we currently know about the combination effects of all the different chemical substances that people are exposed to in their day-to-day lives, it may be wise to apply a principle of caution in this regard. The EU is yet to decide what counts as an endocrine disrupting property, or which methods should be used to test whether a substance is an endocrine disruptor, which also complicates risk assessment (European Commission 2014). A list of risk phrases for the pesticides measured in the family members in the study can be found in Appendix 2, Table 3.

4.4 Uncertainties

In addition to the uncertainties highlighted above in the form of shortcomings in the risk assessment processes where several chemicals interact, we also want to highlight that the study's sample size (five people of different genders and ages) is too small to be able to make any far-reaching conclusions of scientific significance with regard to how significant a change in diet is for exposure to chemicals. Nevertheless the investigation is still interesting as a pilot study, and may constitute a good basis for further investigations.

5   Conclusions

The results of this study indicate that exposure to pesticides reduces when we eat organic products instead of conventionally grown food, and clarifies the significance of food as a source of exposure to chemicals. In this study we have been able to determine that the concentrations of selected pesticides decreased by an average of a factor of 9.5 when the family switched to organic food, which probably means that their total chemical load decreases. In relative terms, the children's load decreased more than the adults' in the food switch, probably due to their higher food intake relative to their body weight. The same exposure to chemicals results in higher concentrations of chemical residues in the bodies of children than in adults (Swedish Chemicals Agency 2014).

Choosing organic foods not only reduces the levels of a number of pesticides that we are exposed to through what we eat, but also reduces the risk of a long-term impact and combination effects.

We also help to reduce the spread of chemicals in the environment, and protect those who work in the cultivation of fruit and vegetables.

Considering that in our day-to-day lives we are exposed to a considerable number of other chemical substances depending on our choices of food, cleaning products, shampoo, furniture, and other items, it is difficult to make a complete assessment of how much the total chemical load decreased. A more comprehensive study in which exposure to a greater number of chemical substances is examined in a greater number of individuals is required in order to make such an assessment.

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Databases

EU Pesticide Database
http://ec.europa.eu/food/plant/pesticides/pesticides_database/index_en.htm
Bekämpningsmedelsregistret

Websites

European Commission 2014 Endocrine Disruptors: What is the existing approach in the European Community?

http://ec.europa.eu/environment/chemicals/endocrine/strategy/euapproach_en.htm

Appendix A. Methodology description

Sampling

All urine samples from the family members were provided in 250 ml bottles made of polypropylene (PP). The samples were frozen in situ in a portable freezer for onward delivery to IVL's laboratory in Stockholm.

Sample processing

CCC, mepiquat, ETU, Propamocarb, MCPA, 2,4-D and Atrazine
Urine samples (6 ml) were spiked with 20 µl of the internal standards aniline-D_5 (1000 ng/ml), carbamazepine-$_{13}C_{15}N$ (1000 ng/ml) and ibuprofen-D_3 (1000 ng/ml). 1.5 g of sodium chloride (NaCl), and 6 mL acetonitrile (ACN) were also added to the sample. The sample was mixed vigorously for 30 seconds and shaken for 30 minutes at 1400 rpm. The supernatant was transferred to an Oasis HLB column (6cc, Waters) conditioned with 6 ml ACN. The supernatant was filtered through the column and into a new test tube. The supernatant was then evaporated to dryness under nitrogen at 40°C. The sample was redissolved in 200 µl of methanol:water (1:1) and centrifuged before it was transferred to vials with 250 µl:s insert for final determination.

3-PBA, TCP, Thiabendazole and Boscalid
Urine samples (6 ml) were spiked with 20 µl of the internal standards aniline-D_5 (1000 ng/ml), carbamazepine-$_{13}C_{15}N$ (1000 ng/ml) and ibuprofen-D_3 (1000 ng/ml). The sample was hydrolysed with 1.0 ml of 10 M sodium hydroxide (NaOH) for 2 hours at 80˚C. After the hydrolysis the sample was allowed to cool to room temperature before 6 ml of methyl tertiary butyl ether (MTBE) was added to the sample. The sample was then mixed vigorously for 30 seconds and shaken for 30 minutes at 1400 rpm. The supernatant was evaporated to dryness under nitrogen at 40°C. The sample was redissolved in 200 µl of methanol:water (1:1) and centrifuged before it was transferred to vials with 250 µl:s insert for final determination.

3,5-DCA
Urine samples (6 ml) were spiked with 20 µl of the internal standard aniline-D_5 (1000 ng/ml). The sample was hydrolysed with 1.0 ml of 10 M NaOH for 2 hours at 80˚C. After the hydrolysis the sample was allowed to cool to room temperature before 6 ml of methyl tertiary butyl ether (MTBE) was added to the sample. The sample was then vortexed for 30 seconds and then shaken for 30 minutes at 1400 rpm. The supernatant was evaporated to dryness under nitrogen at 40°C. The sample was redissolved in 50 µl 50 mM of borate buffer followed by 50 µl 20 mM 3,5-dinotrobenzoyl chloride dissolved in ACN and diluted with 100 µl ACN and transferred to vials with 250 µl:s insert. The sample was incubated for 60 minutes at 60˚C before final determination.

Creatinine
Urine samples were diluted 1000 times with MQ water. 1 ml of the diluted sample was transferred to vials and 50 μl of internal standard carbamazepine-$_{13}$C$_{15}$N (1000 ng/ml) was added before final determination.

Final determination

Analysis of pesticides
The final determination of the amount of pesticides in the samples was performed on a binary liquid chromatography (UFLC) system with auto-injection (Shimadzu, Japan). The chromatographic separation was performed with gradient elution on a C18 reversed phase column (dimensions 150 x 2.1 mm, 3 μm particle size) (Atlantis, Waters) at a temperature of 35°C and a flow of 0.3 ml/minute. The mobile phase consisted of 10 mM acetic acid in water (A) and methanol (B). The gradient was initiated with the composition of 75% A and 25% B, which was kept constant for 1 minute. After 1 minute, the proportion of B was increased evenly to 95% over 11 minutes and kept at 95% for a further 5 minutes. After 1 minute, the proportion of B was reduced to 25% over 1 minute and kept at 25% for a further 3 minutes before a new injection began. The total analysis time was 20 minutes. The UFLC system was linked to an API 4000 triple quadrupole (MS/MS) (Applied Biosystems) with electrospray ionisation interface (ESI) run in positive and negative mode.

Analysis of creatinine
The final determination of the amount of creatinine in the samples was performed on a binary liquid chromatography (UFLC) system with auto-injection (Shimadzu, Japan). The chromatographic separation was performed with gradient elution on a flurophenyl column (dimensions 50 x 2.1 mm, 3 μm particle size) (PFPP, Restek) at a temperature of 35°C and a flow of 0.3 ml/minute. The mobile phase consisted of 10 mM acetic acid in water (A) and methanol (B). The gradient was initiated with the mobile phase composition of 100% A and 0% B. The proportion of B was increased evenly to 95% over 11 minutes and kept at 95% for a further 5 minutes. After 1 minute, the proportion of B was reduced to 0% over 1 minute and kept at 0% for a further 3 minutes before a new injection began. The total analysis time was 20 minutes. The UFLC system was linked to an API 4000 triple quadrupole (MS/MS) (Applied Biosystems) with electrospray ionisation interface (ESI) run in positive mode.

Validation

A sample from each family member was spiked with 100 ng of all studied plant protection products before sample processing commences. These samples underwent the same processing as the samples that were analysed for background levels of plant protection products from food. Apart from these samples, a standard addition was also carried out on four samples from each family member by spiking them with 10, 20, 50 and 100 ng of each plant protection plant protection product once the processing of the samples was complete. The results from the spiking before sample processing and from the standard additions after sample processing were used to estimate the ion suppression and recovery for the analysis methods.

Appendix B. Chemical and toxicological information

Table B1. CAS no., chemical structure and log Kow for the pesticides and degradation products analysed.
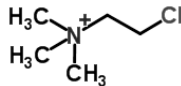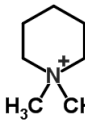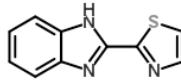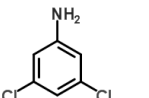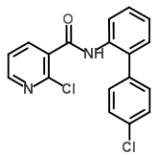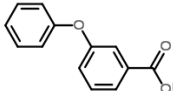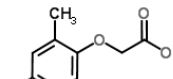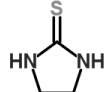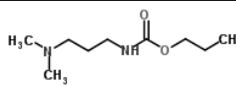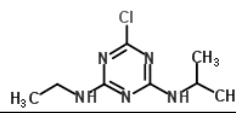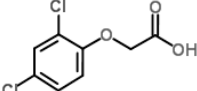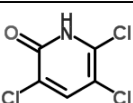
CAS no.	Substance	Abbreviation	Structure	Log Kow
999-81-5	Chlormequat chloride	CCC		-3.8
24307-26-	Mepiqua	MQ		-2.47
148-79-8	Thiabendaz	-		2.88
626-43-7	3,5-	3,5-DCA		2.7
188425-85-6	Boscalid	-		4.31
3739-38-	3-Phenoxybenzoic	3-PBA		3.91
94-74-6	MCPA	MCPA		2.49
96-45-7	Ethylenethio	ETU		-0.66
24579-73-	Propamocarb	-		1.12
1912-24-	Atrazin	-		2.63
94-75-7	2,4-Dichlorophenoxyacetic acid			2.59
6515-38-	3,5,6-trichloro-2-pyridinol			3.38

Table B2. Risk phrases (MSDS) when handling the concentrates of the pesticides measured in the study.

Pesticide	Metabolite	Risk phrases when handling
Ethylenebisdithiocarbamates	ETU	Suspected of damaging unborn children May cause allergic skin reactions Highly toxic to aquatic organisms
Chlorpyrifos	TCP	Toxic if inhaled Highly toxic to aquatic organisms with long-term effects as a result.
For example, iprodione, diuron, vinclozolin	3,5-DCA	Suspected of causing cancer Highly toxic to aquatic organisms with long-term effects as a result.
2,4-D		Harmful if swallowed May cause allergic skin reactions Causes serious eye damage. May cause respiratory irritation Long-term harm to aquatic organisms.
Pyrethroids such as cypermethrin, esfenvalerate	3-PBA	Toxic if inhaled Highly toxic to aquatic organisms with long-term effects as a result. May cause allergic skin reactions Toxic by inhalation
Propamocarb		Harmful if swallowed
Chlormequat chloride (CCC)		Harmful if swallowed Harmful if contact is
Mepiquat		Harmful if swallowed Long-term harm to aquatic organisms.

Appendix C. Analysis results

Table C1. Creatinine-normalised concentration (μg/g crt) of pesticide residues in urine samples and concentration of creatinine from the family members during the experiment periods. As detection limits were set for non-normalised samples, these were different for different samples as they were normalised against creatinine. The degree of recovery (%) for each analysed substance is stated at the bottom.

			CCC	Mepiq uat	ETU	Propa mocarb	2,4-D	3-PBA	TCP	3,5-DC	Creatinine (mg/L)
Period 1	Father	Day 1	<0.6	2.0	<0.3	<0.1	<0.1	5.7	9.7	4.1	1795
		Day 3	2.9	7.4	<0.5	<0.1	1.2	5.5	5.9	22	922
		Day 4	25	15	<1.6	<0.3	<0.7	16	15	<1.3	318
		Day 6	10	4.8	12.2	7.6	<0.3	15	13	1.4	859
	Mother	Day 1	88	23	<1.0	<0.2	<0.5	6.5	32	40	488
		Day 3	<4.3	36	<1.9	<0.4	<0.9	39	40	21	257
		Day 4	404	211	<2.3	<0.5	<1.0	22	18	49	220
		Day 6	323	48	16.5	6.8	<0.6	56	66	69	344
	Girl, 12	Day 1	241	13	<1.9	2.1	20.7	23	44	34	260
		Day 3	57	8.5	<1.9	<0.4	29.5	36	63	60	258
		Day 4	468	60	<2.8	<0.6	29.2	28	91	8.8	177
		Day 6	14	3.4	<0.6	2.5	2.0	12	16	1.5	816
	Girl, 10	Day 1	6.7	2.7	<0.6	<0.1	2.8	20	12	<0.5	866
		Day 3	11	3.1	<1.6	<0.3	6.0	32	17	<1.3	316
		Day 4	16	2.9	<0.8	<0.2	1.9	25	18	1.4	628
		Day 6	13	2.1	<0.9	5.2	2.0	24	30	<0.7	573
	Boy, 3	Day 1	627	36	<3.0	<0.6	4.4	<6.4	31	20	900
		Day 3	675	95	<2.5	5.6	7.2	55	89	18	1120
		Day 4	248	33	<3.4	<0.7	16	63	116	27	799
		Day 6	654	77	<1.8	8.0	2.2	51	50	<1.4	1540
Period 2	Father	Day 1	<0.8	5.2	<0.4	<0.1	<0.2	<0.8	14	<0.3	1300
		Day 3	<1.3	3.5	<0.6	<0.1	<0.3	<1.3	20	<0.5	821
		Day 5	<1.6	5.5	<0.7	<0.1	<0.3	<1.5	34	<0.6	691
		Day 7	<1.3	2.7	<0.6	<0.1	<0.3	<1.2	12	<0.5	876
	Mother	Day 1	<2.5	15	<1.2	<0.2	<0.5	<2.4	15	<0.9	433
		Day 3	<0.8	2.4	<0.4	<0.1	<0.2	<0.8	6.4	<0.3	1360
		Day 5	<1.3	13	<0.6	<0.1	<0.3	<1.3	<1.4	<0.5	834
		Day 7	<3.0	25	<1.4	<0.3	<0.6	<2.9	<3.3	<1.1	365
	Girl, 12	Day 1	9.3	3.6	<0.8	<0.2	<0.3	<1.7	<1.9	<0.6	633
		Day 3	15	<1.3	<0.8	<0.2	4.1	<1.6	<1.9	<0.6	638
		Day 5	<2.5	<1.9	<1.1	<0.2	<0.5	<2.3	<2.7	<0.9	447
		Day 7	<1.1	<0.8	<0.5	<0.1	0.9	<1.0	<1.2	<0.4	1005
	Girl, 10	Day 1	<1.3	<1.0	<0.6	<0.1	1.1	<1.3	<1.4	<0.5	828

		Day 3	<1.6	<1.2	<0.7	<0.1	1.7	<1.5	<1.7	<0.6	694
		Day 5	<5.1	<4.0	<2.3	<0.5	<1.0	<4.9	<5.6	<1.9	215
		Day 7	<1.2	<0.9	<0.5	0.6	1.7	<1.1	<1.3	<0.4	944
	Boy, 3	Day 1	<4.9	<3.8	<2.2	<0.4	<1.0	<4.6	<5.3	<1.8	226
		Day 3	<3.4	8.5	<1.5	<0.3	<0.7	<3.2	<3.7	<1.2	327
		Day 5	<12	<9.5	<5.5	<1.1	<2.4	<12	<13	<4.5	90
		Day 7	<3.5	<2.7	<1.6	<0.3	<0.7	<3.3	<3.8	<1.3	316
Recovery	(%)		60	62	39	49	65	73	71	101	

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Appendix D. Estimates of daily intake

The estimated daily intake (EDI) was calculated as follows (Mage et al. 2004):

$$EDI(\mu g\ kg^{-1}day^{-1}) = \frac{UE_{crea}(\mu g\ g\ crea^{-1}) \times CE_{smoothed}\ (g\ day^{-1})}{BW\ (kg)} \quad\quad [1]$$

where

$$UE_{crea}(g\ g_{crea}^{-1}) = \frac{UC(\mu g\ L^{-1}) \times 1000(mg\ g^{-1})}{UC_{crea}\ (mg\ L^{-1})} \quad\quad [2]$$

UC is the concentration of pesticides in the urine, UC_{crea} is the concentration of creatinine in the urine (see Table 3, Appendix B) and the UE_{crea} is the creatinine-normalised concentration of pesticides in the urine.

BW in equation [1] is body weight in kg and $CE_{smoothed}$ is the rate of excretion of creatinine per day. This was determined according to Mage et al., 2004:

$$CE_{smoothed}(g\ day^{-1}) = A \times (140 - Age\ (years)) \times BW_{1.5} \times Height\ (cm)_{0.5} \quad\quad [3]$$

Where A = 1.93 for men and 1.64 for women.

For children $CE_{smoothed}$ (g day^{-1}) was calculated from Remer et al. (2002) as follows:

$$CE_{smoothed}(g\ day^{-1}) = CE(mmol\ kg^{-1}day^{-1} \times BW\ (kg) \times MW_{crea}\ (g\ mmol^{-1}) \quad\quad [4]$$

Where CE = 0.183 for girls (9-13 years) and 0.131 for the 3-year-old boy, all according to Remer et al. (2002)

CEsmoothed calculated as above is:

Father: 2.53
Mother: 1.67
Girl, 12:   0.93
Girl, 10:   0.93
Boy, 3:   0.22